



# Plasma eotaxin level and severity of asthma treated with corticosteroid

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## KEYWORDS

Eotaxin;  
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IL-5

**Summary** Our understanding of asthma severity was advanced by the identification of biomarkers which account for differences in lung function impairment. We tried to examine the effects of corticosteroid treatment on known correlates of asthma severity including peripheral eosinophil counts, total IgE, IL-5, and eotaxin levels in plasma.

We compared these biomarkers among groups of stable asthmatics categorized by the dose of corticosteroid (N: steroid-free,  $n = 25$ ; L: low-dose inhaled,  $n = 27$ ; MH: medium or high-dose inhaled,  $n = 19$ ; O: inhaled plus oral,  $n = 8$ ). Next we compared these markers and peak expiratory flow rate (PEFR) in unstable asthmatics before and after treatment with steroids ( $n = 22$ ).

Eotaxin levels in the O group were higher than those in the N and MH groups ( $P < 0.05$ ). Logistic regression analysis demonstrated that plasma eotaxin level was correlated with the severity of asthma defined by treatment intensity ( $P = 0.01$ ) and % FEV<sub>1</sub> ( $P = 0.04$ ) while the other markers were not. Eotaxin levels did not change after steroid treatment in unstable patients, whereas eosinophil counts decreased in parallel with PEFR.

Among biomarkers of asthma severity studied, plasma eotaxin level was not significantly affected by corticosteroid treatment, and was associated with the severity even in the presence of steroid therapy.

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## Introduction

Bronchial asthma is a chronic disease characterized by eosinophilic inflammation of the airways. Since the introduction of inhaled steroid therapy, asthma treatment has dramatically improved and most asthmatics have been managed in outpatient clinics

without hospital admission. The concept of asthma severity has also changed and the required dose of inhaled steroid to control clinical symptoms and maintain pulmonary function has been regarded as one of the important indices of disease severity.<sup>1</sup> Inhaled corticosteroid therapy has been thought to cause few adverse effects, but there is accumulating evidence that long-term and high-dose therapy may lead to unfavorable effects including cataracts and osteoporosis.<sup>2,3</sup> It is, therefore, important to determine the optimal dose of inhaled steroid to maintain long-term remission in asthma patients. In

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addition, some severe asthmatics do not respond well to inhaled steroids and require continuous systemic steroid therapy. It is thus meaningful to associate predictive biomarkers with asthma severity as judged by intensity of treatment.

Previous reports demonstrated that various parameters such as peripheral eosinophil counts,<sup>4,5</sup> IgE levels,<sup>6,7</sup> IL-5 levels,<sup>8,9</sup> and %FEV<sub>1</sub> are useful markers for monitoring the activity or predicting the severity of asthma. However, these parameters may be modified by treatment with corticosteroids, and their usefulness has not been fully analyzed in the presence of steroid therapy although the majority of asthmatics are now being treated with steroids. Eotaxin is a CC chemokine that recruits eosinophils by activating the CCR3 receptor.<sup>10</sup> The expression of eotaxin was found to be increased in the airways of asthmatics, and the elevation of eotaxin levels was proportional to eosinophil infiltration and bronchial hyperreactivity.<sup>11,12</sup> We have recently reported that plasma eotaxin levels inversely correlate with lung function in asthmatics not treated with steroids.<sup>13</sup> In addition, plasma eotaxin levels were reported to increase in patients with acute asthma compared with those in stable patients.<sup>14</sup> It was also suggested that plasma eotaxin level tended to be higher in severe asthmatics than in mild patients when they were stratified by the response to emergency treatment, the presence of systemic steroid on presentation, or the requirement of hospital admission.<sup>14</sup> Based on these observations we hypothesized that plasma eotaxin level can be a biomarker of asthma severity regardless of steroid treatment. In order to test this hypothesis, we compared plasma eotaxin levels, peripheral eosinophil counts, IL-5 levels, IgE levels, and %FEV<sub>1</sub> among patients with stable asthma of different severity based on the intensity of steroid treatment. Next we compared these markers in symptomatic asthmatics before and after treatment with steroids.

## Materials and methods

### Subjects

One hundred and one asthma patients visiting the outpatient clinic at Keio University Hospital between August and September, 1998 (79 stable patients) and between April and July, 2000 (22 unstable patients) were enrolled. All patients met the diagnostic criteria for asthma in the Global Initiative for Asthma,<sup>1</sup> and had been diagnosed with asthma by a physician at least 1 year before enrollment in the present study.

In order to investigate the differences in biomarkers in stable asthmatics of different severity, consecutive patients who met the following criteria were designated as stable patients; (1) medication was unchanged at least for 3 months, and (2) step-up of treatment was judged unnecessary from their clinical symptoms and pulmonary function, i.e., they had minimal chronic symptoms including nocturnal symptoms, minimal exacerbations, no emergency visits, minimal use of as-needed  $\beta_2$ -agonist, no limitations on activities, and FEV<sub>1</sub> greater than 80% of the personal best.<sup>1</sup> The enrolled asthma patients were divided into four groups according to the maintenance doses of corticosteroids; (1) N group,  $n = 25$ : not treated with steroids, (2) L group,  $n = 27$ : treated with a low dose of inhaled beclomethasone dipropionate (BDP) ( $\leq 500 \mu\text{g/day}$ ),<sup>1</sup> (3) MH group,  $n = 19$ : treated with a medium or high dose of inhaled BDP ( $> 500 \mu\text{g/day}$ ), (4) O group,  $n = 8$ : treated with both a medium or high dose of inhaled BDP and oral daily prednisolone (PSL). BDP was inhaled with a spacer in all patients. Stable asthmatics were also divided into three groups by %FEV<sub>1</sub>; (1) mild group,  $n = 45$ : %FEV<sub>1</sub>  $\geq 80\%$ , (2) moderate group,  $n = 19$ :  $60\% \leq \text{\%FEV}_1 < 80\%$ , (3) severe group,  $n = 13$ : %FEV<sub>1</sub>  $< 60\%$ . In two patients baseline pulmonary function test was not available and they were judged as stable by their clinical symptoms.

To elucidate the effects of steroids on clinical parameters during an acute exacerbation of asthma, consecutive patients who met the following criteria were defined as unstable; (1) step-up or additional treatment was necessary based on their clinical symptoms and pulmonary function, and (2) medication was unchanged for at least 3 months prior to initiating additional therapy with steroids. There were no patients who required admission or intravenous steroid therapy during the study period. No patient overlapped between the stable and unstable groups. The unstable patients were categorized into three groups in terms of steroid therapy; (1) N-US [fluticasone propionate (FP)],  $n = 9$ : unstable asthmatics who had not been treated with steroids, and initiation of inhaled steroid therapy was judged necessary. These patients were treated with  $200 \mu\text{g}$  FP bid for 4 weeks with a disk inhaler. (2) N-US (PSL),  $n = 6$ : unstable asthmatics who had not been treated with steroids, and oral steroid therapy was considered necessary due to their severe symptoms. These patients were treated with  $30 \text{ mg}$  PSL/day orally for 1 week. (3) IS-US (PSL),  $n = 7$ : unstable asthmatics who had been treated with inhaled steroids (FP:  $200\text{--}800 \mu\text{g/day}$ ), and step-up or additional steroid therapy was considered to be necessary. These

patients were treated with 30 mg PSL/day orally for 1 week regardless of the severity of the exacerbation. No other medication was changed during treatment with steroid. The reason for the change in inhaled steroid from BDP to FP is that FP became available in our hospital in April 1999.

Forty-one healthy subjects without asthmatic manifestations were selected as controls (CTL). Their age and gender were  $30 \pm 3$  (median: 31, range: 24–34) years old and male/female (M/F): 20/21, respectively. Eight subjects were current smokers, three were ex-smokers (who had quit smoking for at least 6 months), and thirty had never smoked. Atopic status was not considered as the inclusion criteria for the CTL. Prior informed consent was obtained from all patients and healthy controls.

### Study design

A cross-sectional study was performed to compare plasma eotaxin and IL-5 levels, serum IgE levels, and peripheral eosinophil counts in stable asthmatics stratified according to their treatment. An open intervention study was also performed to investigate the effects of steroid treatment on the levels of these biomarkers in unstable asthmatics.

### Determination of biochemical and functional parameters

Plasma eotaxin levels were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) as previously reported.<sup>13–15</sup> Plasma IL-5 levels were measured using an ELISA kit purchased from R&D Systems (Minneapolis, MN, USA). Serum IgE level was measured by a chemiluminescent enzyme immunoassay (LUMIWARD, Shionogi, Osaka, Japan). Normal range of serum IgE level was less than 250 IU/ml in this assay. Pulmonary function tests were performed using a spirometer (rolling-seal spirometer, Chest, Tokyo, Japan) when the patients were judged to be clinically stable (77 stable patients) or just after treatment with steroids (22 unstable patients). Peak expiratory flow rate (PEFR) measured in the morning with a peak flow meter (Personal Best, Healthscan Products Inc., Cedar Grove, NJ, USA) was compared before and after steroid therapy with FP (4 weeks) or PSL (1 week) in unstable asthmatics.

### Statistical analysis

Data were expressed as mean  $\pm$  SD and median values. Kruskal–Wallis rank test was used to

compare the values among the N, L, MH, and O groups and Dunnett test was applied as a post-hoc test. Mann–Whitney's *U*-test was performed to compare the values between two unpaired groups. Wilcoxon signed-ranks test was used to compare values between two paired groups. Multiple logistic regression analysis was performed to examine the contribution of factors to asthma severity. A *P*-value of less than 0.05 was considered statistically significant.

## Results

### Clinical profiles of asthma patients

The clinical characteristics of stable and unstable asthmatics in each group are presented in Table 1. All patients were treated with inhaled or oral  $\beta$ -agonists. Some asthmatics were treated with theophylline and/or a cysteinyl leukotriene 1 receptor antagonist. There was no statistically significant difference in %FEV<sub>1</sub> among the N, L, MH, and O groups or between the N-US and IS-US groups.

### Plasma eotaxin level and peripheral eosinophil count in steroid-free and steroid-treated asthmatics

Plasma eotaxin levels were higher in asthmatics treated with inhaled steroids (STEROID group: *n* = 61, patients in the L, MH, O, and IS-US groups) than in asthmatics who had not required continuous steroid therapy (NONE group: *n* = 40, patients in the N and N-US groups) (\**P* < 0.05,  $267 \pm 193$ , 218 vs.  $190 \pm 153$ , 165 pg/ml, respectively) (Fig. 1a). There was no significant difference in eosinophil count between the STEROID and NONE groups ( $425 \pm 301$ , 347 vs.  $437 \pm 348$ , 367  $\mu$ l, respectively) (Fig. 1b). Neither was there a difference in IgE level between the two groups (*P* = 0.15,  $750 \pm 1160$ , 380 vs.  $660 \pm 1380$ , 180 IU/ml, respectively). In contrast to the behavior of eotaxin, plasma IL-5 level in the STEROID group was lower than that in the NONE group (*P* = 0.046,  $1.2 \pm 3.1$ , 0 vs.  $2.3 \pm 3.2$ , 0 pg/ml), although the mean level was below the detection limit in both groups. In the N-US and IS-US groups, only the values before initiating steroid therapy were included in this analysis.

When the STEROID and NONE groups were combined, eotaxin levels were significantly higher in the asthmatics group (*n* = 101) than in the CTL group (*P* < 0.0001,  $237 \pm 181$ , 190 vs.  $121 \pm 110$ , 108 pg/ml, respectively). IgE levels were also higher

**Table 1** Clinical profiles of asthmatics.

Group	n	Gender (M/F)	Age	Duration (years)	Smoking (C/E/NS)	Inhaled steroid ( $\mu$ g/day)	Oral PSL (mg/day)	%FEV <sub>1</sub>	Total IgE (IU/ml)
N	25	16/9	51 $\pm$ 17	14 $\pm$ 18	6/6/13	0	0	87 $\pm$ 20 (89)	720 $\pm$ 1650 (173)
L	27	12/15	51 $\pm$ 16	14 $\pm$ 10	3/3/21	BDP 290 $\pm$ 100	0	83 $\pm$ 15 (83)	790 $\pm$ 1290 (375)
MH	19	12/7	53 $\pm$ 16	13 $\pm$ 15	3/7/9	BDP 1070 $\pm$ 490	0	84 $\pm$ 21 (89)	770 $\pm$ 1220 (369)
O	8	3/5	65 $\pm$ 10	16 $\pm$ 14	2/2/4	BDP 1600 $\pm$ 430	7.2 $\pm$ 3.1	68 $\pm$ 29 (61)	670 $\pm$ 1050 (380)
N-US	15	10/5	45 $\pm$ 16	8 $\pm$ 10	4/2/9	0	0	86 $\pm$ 16 (86)	560 $\pm$ 750 (195)
IS-US	7	3/4	48 $\pm$ 11	19 $\pm$ 19	1/1/5	FP 400 $\pm$ 200	0	80 $\pm$ 21 (81)	630 $\pm$ 620 (405)

M: male; F: female; C: current smoker; E: ex-smoker; NS: nonsmoker; PSL: prednisolone; N: clinically stable asthmatics not treated with steroid; L: those with low-dose inhaled steroid; MH: those with medium or high-dose inhaled steroid; O: those with medium or high-dose inhaled steroid and oral PSL; N-US: unstable asthmatics not treated with steroid; IS-US: those with inhaled steroid; BDP: beclomethasone dipropionate; FP: fluticasone propionate. Parentheses represent median values of %FEV<sub>1</sub> and total IgE level.

in the asthmatics group than in the CTL group ( $P < 0.01$ ,  $720 \pm 1240$ , 330 vs.  $200 \pm 190$ , 130 IU/ml, respectively). Peripheral eosinophil counts and IL-5 levels were not available in the CTL group. In the asthmatics group ( $n = 101$ ), IL-5 levels were significantly correlated with eosinophil counts ( $r = 0.21$ ,  $P = 0.03$ ) while neither eotaxin nor IgE was correlated with them.

### Relation of biomarkers to intensity of asthma treatment

Plasma eotaxin levels in the N, L, MH, O, N-US, and IS-US groups were  $181 \pm 164$  (157),  $259 \pm 216$  (177),  $196 \pm 145$  (190),  $390 \pm 171$  (421),  $206 \pm 136$  (177), and  $351 \pm 171$  (344) pg/ml, respectively. Eotaxin levels were increased in the O group compared to the N and MH groups ( $*P < 0.05$ , Fig. 2a). Eotaxin levels in the IS-US group were higher than in the N-US and MH groups ( $^{\dagger}P < 0.05$ , Fig. 2a).

Peripheral eosinophil counts in the N, L, MH, O, N-US, and IS-US groups were  $378 \pm 236$  (322),  $391 \pm 253$  (303),  $584 \pm 350$  (551),  $125 \pm 71$  (106),  $535 \pm 474$  (403), and  $467 \pm 219$  (451)  $\mu$ l<sup>-1</sup>, respectively. Eosinophil counts were increased in the MH group compared to that in the N, L, and O groups ( $*P < 0.05$ , 0.05, and 0.01, respectively, Fig. 2b).

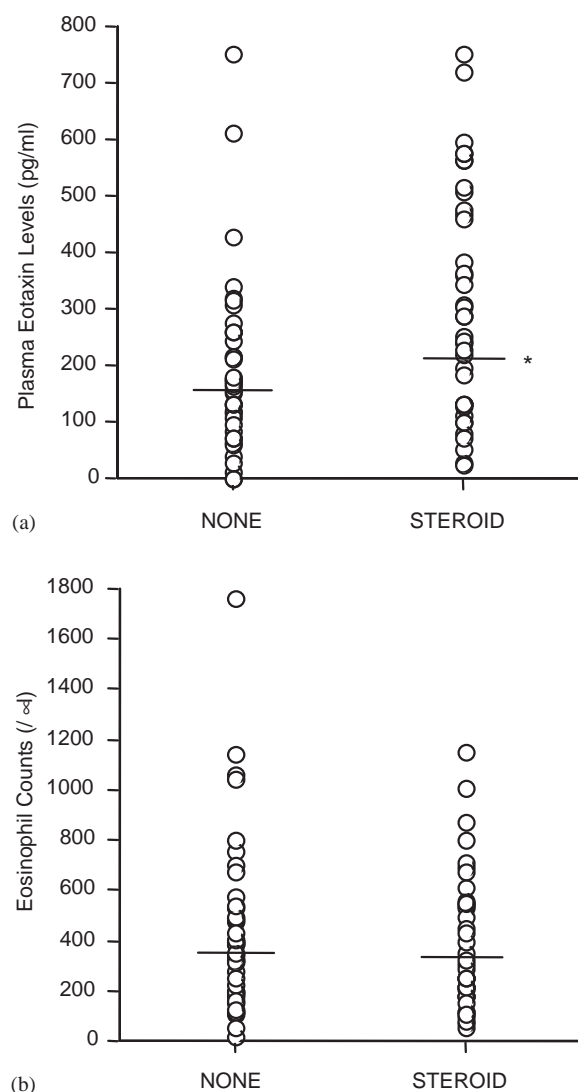
There was no significant difference in IgE levels among the N, L, MH, and O groups or between the N-US and IS-US groups (Table 1). IL-5 levels were higher in the N-US group than in the N group [ $3.7 \pm 3.3$  (2.3) vs.  $1.4 \pm 0.6$  (0) pg/ml,  $P < 0.01$ ].

### Biomarkers and FEV<sub>1</sub>

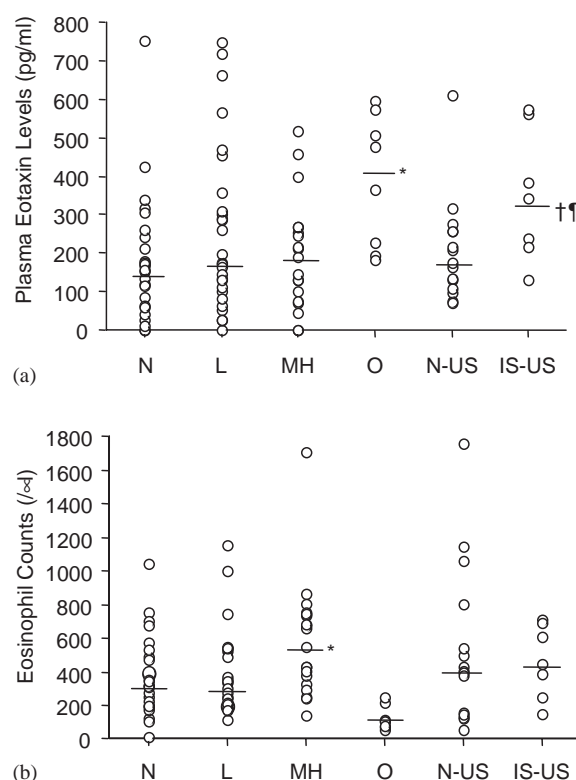
Plasma eotaxin levels in the mild, moderate, and severe groups classified by %FEV<sub>1</sub> were  $230 \pm 26$  (201),  $205 \pm 46$  (130), and  $298 \pm 61$  (268), respectively. The difference in eotaxin levels among the groups was not statistically significant. Neither was there a significant difference in plasma IL-5 levels, serum IgE levels, or peripheral eosinophil counts among the groups.

### Logistic regression analysis of factors related to severity of asthma

Factors independently correlated with the severity of asthma were evaluated by multiple logistic regression analyses in stable asthmatics ( $n = 79$ ) (Table 2). Severe asthma was defined as belonging to the O group or %FEV<sub>1</sub>  $< 60$  in these analyses. Since the negative effect of oral steroids on peripheral eosinophil counts and IL-5 levels were apparent, these factors were excluded from the analysis of the O group. Plasma eotaxin level and age were correlated with the severity judged by treatment intensity. These two factors were also correlated with the severity evaluated by pulmonary function. The other factors did not significantly relate to the severity defined by these two criteria. There was no correlation between age and plasma eotaxin levels in asthmatics ( $r = 0.10$ ,  $P = 0.37$ ) or in control subjects ( $r = 0.03$ ,  $P = 0.84$ ) by a simple linear regression analysis.



**Figure 1** Plasma eotaxin levels (a) and peripheral eosinophil counts (b) in asthmatics treated with (STERIOD:  $n = 61$ ) or without steroids (NONE:  $n = 40$ ). Horizontal lines represent median values. \* $P < 0.05$ , compared with NONE.



**Figure 2** Plasma eotaxin levels (a) and peripheral eosinophil counts (b) in asthmatics. N: stable patients not treated with steroid, L: stable patients treated with low-dose inhaled steroid, MH: stable patients treated with medium or high-dose inhaled steroid, O: stable patients treated with inhaled and oral steroids, N-US: unstable patients not treated with steroid, IS-US: unstable patients treated with inhaled steroids. Horizontal lines represent median values. Eotaxin level was higher in the O group than in the N and MH groups (a:  $P < 0.05$ ). \* $P < 0.05$  vs. the N-US group, † $P < 0.05$  vs. the MH group (a). Eosinophil count was higher in the MH group than in the N, L (\* $P < 0.05$ ), and O (\* $P < 0.01$ ) groups (b).

**Table 2** Logistic regression analysis of factors related to severity of asthma.

	O group			%FEV < 60		
	$\chi^2$	P	OR (95%CI)	$\chi^2$	P	OR (95%CI)
Age (10 years)	6.3	0.01	4.7 (1.4–15.8)	8.1	0.01	2.8 (1.4–5.5)
Smoking history	2.3	0.13	5.3 (0.6–46.1)	0.01	0.94	0.9 (0.2–4.1)
Eotaxin (100 pg/ml)	7.3	0.01	2.5 (1.3–4.9)	4.0	0.04	1.5 (1.0–2.3)
Eosinophil (100/ $\mu$ l)	—	—	—	0.9	0.35	1.1 (0.9–1.5)
IgE (100 IU/ml)	2.6	0.10	1.1 (1.0–1.2)	0.02	0.89	1.0 (0.9–1.1)
IL-5 (1 pg/ml)	—	—	—	0.4	0.54	1.1 (0.9–1.3)

O group: asthmatics treated with inhaled and oral steroids; OR: odds ratio; CI: confidence interval.



**Table 3** Biomarker levels in stable and unstable asthmatics.

	<i>n</i>	IL-5 (pg/ml)	Eotaxin (pg/ml)	Eosinophil count ( $\mu\text{l}^{-1}$ )
Stable asthmatics	79	$1.26 \pm 3.05$ (0)	$232 \pm 188$ (179)	$406 \pm 289$ (326)
Unstable asthmatics	22	$2.91 \pm 3.33$ (1.61 <sup>*</sup> )	$252 \pm 159$ (216)	$513 \pm 406$ (417)

<sup>\*</sup> $P < 0.01$ . Parentheses represent median values.

### Effects of treatment with steroids on biomarker levels in unstable asthmatics

Biomarker levels in stable and unstable asthmatics are shown in Table 3. IL-5 level was significantly higher in unstable asthmatics than in stable patients. Eotaxin level and eosinophil counts were not significantly different between the groups. Changes in biomarker levels and PEFR before and after treatment with FP or PSL in unstable asthmatics are presented in Table 4. Eotaxin levels did not change after treatment with inhaled FP in the N-US (FP) group, or after treatment with oral PSL in the N-US (PSL) and the IS-US (PSL) groups. PEFR significantly increased after treatment with FP or PSL in the N-US (FP), N-US (PSL), and IS-US (PSL) groups, despite the behavior of eotaxin. In contrast, peripheral eosinophil counts tended to decrease after treatment with FP in the N-US (FP) group ( $P = 0.07$ ) and significantly decreased after treatment with PSL in the N-US (PSL) and the IS-US (PSL) groups ( $P = 0.03$  and  $0.02$ , respectively) (Table 4). There was no significant change in IgE levels before and after the treatment in any of the groups studied. IL-5 levels were decreased after steroid treatment in the N-US (PSL) group. The ratio of PEFR after steroid treatment to that before the treatment was significantly correlated with plasma eotaxin levels before the treatment in N-US(FP) and IS-US(PSL) groups (Fig. 3). In N-US(PSL) group there was an exceptional patient with low eotaxin level and high PEFR ratio (shown as a solid square). When this patient was excluded, there would be a good correlation in this group as well ( $r = 0.92$ ,  $P = 0.02$ ).

### Discussion

The present study demonstrated that plasma eotaxin levels were highest in asthmatics who required both inhaled and systemic steroids. Plasma eotaxin levels were also significantly higher

in asthmatics treated with steroids than in steroid-free asthmatics. Logistic regression analysis suggested that among biomarkers studied only plasma eotaxin levels were related to the degree of airflow obstruction as well as treatment intensity. Treatment of unstable asthmatics with oral or inhaled corticosteroids did not significantly affect plasma eotaxin levels. These observations imply that plasma eotaxin levels are relatively resistant to corticosteroid treatment and is a better marker of asthma severity in steroid-treated asthmatics than biomarkers such as eosinophil counts or IL-5 levels that are affected by this treatment.

In vitro studies confirmed that corticosteroids decrease cytokine-induced eotaxin expression in human epithelial and mononuclear cells in a concentration-dependent manner.<sup>15,16</sup> The present study, however, demonstrated that the systemic expression of eotaxin in vivo is not significantly decreased by clinical doses of inhaled or oral steroids (Table 4). These discrepancies may be accounted for by the different environment between in vitro and in vivo situations, i.e., other unspecified confounding factors augmenting eotaxin expression or interfering with steroid effects may exist in vivo. Plasma eotaxin levels in acute asthma treated with oral corticosteroids also tended to be higher than those not treated with them in a previous study.<sup>14</sup> It is unlikely that systemic steroids increase plasma eotaxin levels partly because plasma eotaxin levels did not significantly change after treatment with oral PSL in healthy volunteers (data not shown).

Peripheral eosinophil counts were correlated with treatment intensity only in the absence of systemic steroids. Correlation between eosinophil counts and severity of asthma was previously demonstrated in asthmatics not treated with steroids.<sup>4</sup> Plasma eotaxin levels tended to increase in asthmatics with acute exacerbations (Fig. 2), which is consistent with the previous observation in acute asthma.<sup>14</sup> However, eotaxin levels did not parallel the improvement in pulmonary function after treatment with steroids, whereas peripheral

**Table 4** Changes in parameters of unstable asthmatics before and after treatment with steroid.

Group	Eotaxin (pg/ml)			Eosinophil ( $\mu\text{l}^{-1}$ )			Total IgE (IU/ml)			IL-5 (pg/ml)			PEFR (l/min)		
	OW	1W	4W	P	OW	1W	4W	P	OW	1W	4W	P	OW	1W	4W
N-US(FP) n = 9	176 ± 79 (163)	NE	164 ± 98 (140)	0.44	583 ± 543 (403)	NE	399 ± 285 (239)	0.07	379 ± 545 (99)	3.9 ± 3.8 (2.4)	NE	2.7 ± 2.4 (2.8)	0.40	420 ± 143 (440)	503 ± 157 (525)
N-US(PSL) n = 6	252 ± 194 (237)	416 ± 482 (213)	NE	0.07	463 ± 386 (448)	136 ± 97 (118)	NE	0.03	800 ± 971 (325)	3.4 ± 2.8 (2.0)	0.4 ± 0.6 (0)	NE	0.04	248 ± 154 (228)	442 ± 164 (455)
IS-US(PSL) n = 7	351 ± 171 (344)	463 ± 328 (355)	NE	0.40	467 ± 219 (451)	119 ± 180 (143)	NE	0.02	632 ± 618 (405)	1.3 ± 3.0 (0)	1.8 ± 3.0 (0.6)	NE	0.89	314 ± 159 (380)	396 ± 182 (392)

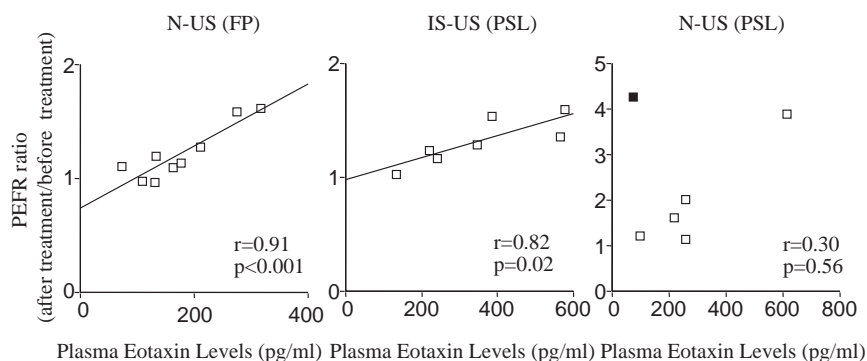
PEFR = peak expiratory flow rate; W = weeks after initiating steroid therapy; FP = fluticasone propionate; N-US (FP) = unstable asthmatics who had not been treated with steroid and were treated with FP for 4 weeks; PSL = prednisolone; N-US (PSL) = unstable asthmatics who had not been treated with steroid and were treated with PSL for 1 week; IS-US = unstable asthmatics who had been treated with inhaled steroid and were treated with PSL for 1 week; NE = not examined. Parentheses represent median values.

eosinophil counts were closely related to the improvement in pulmonary function after initiating steroid therapy. These results also correspond to the previous finding that eotaxin levels were elevated a week after the onset of symptoms of an asthma exacerbation,<sup>14</sup> suggesting that the elevated eotaxin levels during an exacerbation continue for at least several days.

In addition, there was a significant correlation between the pretreatment levels of plasma eotaxin and the extent of functional improvement after steroid treatment in unstable asthmatics (Fig. 3). These results suggest that plasma eotaxin level is a marker of disease activity and/or treatment response to steroids in unstable asthma. Taken together, plasma eotaxin levels may reflect acute exacerbations of asthma as well as baseline severity even in the presence of steroid treatment, while peripheral eosinophil counts are promptly suppressed by treatment with steroid. However, there was no significant difference in eotaxin levels between all stable and unstable asthmatics (Table 3). These observations may be attributable to the difference in the baseline severity between the stable and unstable patients (Table 1).

A recent study demonstrated that low-dose BDP (336  $\mu\text{g}/\text{day}$ ) significantly improved lung function of mild-to-moderate asthmatics in the absence of a reduction in clinical markers for eosinophilic inflammation.<sup>17</sup> Another study also suggested that eosinophil counts and eosinophil cationic protein levels were increased in induced sputum of severe asthmatics even in the presence of high-dose inhaled or oral steroids.<sup>18</sup> These observations are not inconsistent with our finding that eosinophil-related inflammation, which was monitored by plasma eotaxin levels, peripheral eosinophil counts, and plasma IL-5 levels in the present study, was not always diminished by inhaled or oral corticosteroid therapy even in asthmatics with stable clinical symptoms.

Plasma IL-5 levels were higher in asthmatics with acute exacerbations than those in stable asthmatics (Table 3). However, IL-5 levels during acute exacerbations decreased after treatment with oral steroids in patients who had not been treated with steroids (N-US group) (Table 4). Plasma IL-5 levels were also lower in asthmatics treated with steroids than those in steroid-free patients. These observations imply that plasma IL-5 levels may parallel the disease activity but are affected by treatment with steroids, as reported in previous studies.<sup>8,19</sup> It is interesting that plasma eotaxin and IL-5 levels were differentially regulated, and IL-5, but not eotaxin, was correlated with eosinophil counts in asthmatics, although both eotaxin and IL-5 are thought



**Figure 3** Relationship between plasma eotaxin levels and the PEFR ratio (after treatment/before treatment) in unstable asthmatics. N-US(FP): unstable patients who had not been treated with steroid and were treated with FP for 4 weeks, IS-US(PSL): unstable patients who had been treated with inhaled steroid and were treated with PSL for 1 week, N-US(PSL): unstable patients who had not been treated with steroid and were treated with PSL for 1 week. A solid square represents an outlying patient with low eotaxin level and high PEFR ratio.

to be paramount in promoting eosinophilic inflammation in allergic diseases.<sup>20</sup> Correlation between serum IgE levels and asthma severity was mainly demonstrated in children with asthma not treated with steroids.<sup>6,7</sup> The effects of steroids on serum IgE levels are influenced by the route of administration, doses of corticosteroids, and duration of treatment and the interpretation of serum IgE levels of asthmatics is controversial in the presence of steroid therapy.<sup>21–23</sup> The present study found that serum IgE levels were not related to either disease severity, activity, or treatment response to steroids in an asthmatic population.

Asthma severity is predicted by biomarkers such as eosinophil counts and IgE levels but disease treatment may effect the levels of these markers. Interpretation of plasma eotaxin level in the assessment of asthma severity is not always easy, because correlation between eotaxin level and asthma severity was not perfect, e.g., there was no significant difference in eotaxin level among the N, L, and MH groups. Our findings, however, demonstrate that among biomarkers which have a predictive value of disease severity, plasma eotaxin level is less effected by treatment with corticosteroids and provides more information about asthma severity that was not available with the other biomarkers after initiation of steroid treatment.

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